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(54) CAMEL, an alternative translation product of the tumour antigen LAGE-1

(57) Tumor-associated antigens and DNAs encoding them. The tumor-associated antigens are encoded by an open reading frame of the LAGE-1 gene. The tumor-associated antigens, immunogenic (poly) pep-

tides derived therefrom and DNAs encoding them, are useful for cancer immunotherapy.

Protein Translations

ORF3

LAGE-1 <sup>a</sup>	MQAECQCTGGSTGADGPGGPGI PDGPGGNAGGPGGACATGGRGPRGAGAAASGPRGCAFRGPGGGAASADGRCPCGA	80
LAGE-1 <sup>b</sup>	MQAECQCTGGSTGADGPGGPGI PDGPGGNAGGPGGACATGGRGPRGAGAAASGPRGCAFRGPGGGAASADGRCPCGA	80
NY-ESO-1	MQAECQCTGGSTGADGPGGPGI PDGPGGNAGGPGGACATGGRGPRGAGAAASGPRGCAFRGPGGGAASADGRCPCGA	80
LAGE-1 <sup>a</sup>	RRPDSRLQLHITMPFSSPHEAELVRRILSRDAAPLPRPGAVLKDFTVSGNLLFIRLTAADHRQLQLSISSCLQQLSLIM	160
LAGE-1 <sup>b</sup>	RRPDSRLQLHITMPFSSPHEAELVRRILSRDAAPLPRPGAVLKDFTVSGNLLFIRLTAADHRQLQLSISSCLQQLSLIM	160
NY-ESO-1	RRPDSRLQLHITMPFSSPHEAELVRRILSRDAAPLPRPGAVLKDFTVSGNLLFIRLTAADHRQLQLSISSCLQQLSLIM	160
LAGE-1 <sup>a</sup>	WITQCFLPVFLAQPSSGQR	180 aa, 18.2 kD
LAGE-1 <sup>b</sup>	WITQCFLPVFLAQPSSGQR	210 aa, 21.1 kD
NY-ESO-1	WITQCFLPVFLAQPSSGQR	180 aa, 18.2 kD

ORF1

LAGE-1 <sup>a</sup>	MLHAGKALAFVMAQGAMLAQERRVPRAAEVPGAQGGQGPGRGEEAPRGVMAVPLLRMEGAPAGPGGRTAACFSCSTR	80
LAGE-1 <sup>b</sup>	MLHAGKALAFVMAQGAMLAQERRVPRAAEVPGAQGGQGPGRGEEAPRGVMAVPLLRMEGAPAGPGGRTAACFSCSTR	80
NY-ESO-1	MLHAGKALAFVMAQGAMLAQERRVPRAAEVPGAQGGQGPGRGEEAPRGVMAVPLLRMEGAPAGPGGRTAACFSCSTR	58
LAGE-1 <sup>a</sup>	CLSRPFWKRSWSAGSCPGMHLSPDQGRF	109 aa, 11.7 kD
LAGE-1 <sup>b</sup>	CLSRPFWKRSWSAGSCPGMHLSPDQGRF	109 aa, 11.7 kD
NY-ESO-1	CLSRPFWKRSWSAGSCPGMHLSPDQGRF	58 aa, 6.2 kD

Fig. 2B

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## Description

[0001] The present invention relates to the field of cancer therapy, more specifically to tumor-associated antigens.

[0002] Cytotoxic T lymphocytes (CTLs) play an important role in the defense against melanoma. Melanoma-specific CTL clones have been obtained from either tumor infiltrating lymphocytes (TIL) *in vitro* stimulated with cytokines, or from peripheral blood mononuclear cells (PBMC) cultured with (autologous) tumor cells. T cell responses against tumor cells are enhanced by cytokine transfection of the tumor cells, both in animal models and in *in vitro* human culture systems. (van Elsas et al., 1997; Gansbacher et al., 1990; Tepper et al., 1989; Fearon et al., 1990; Dranoff et al., 1993)

[0003] The antigens recognized by the tumor-specific T cells become better defined by the development of molecular cloning techniques. These T cell targets can be divided in three groups: 1) tumor-specific antigens, not expressed in healthy tissues, except testis and placenta (e.g., MAGE, BAGE, GAGE), 2) antigens that are lineage-specific and expressed in both melanoma and melanocytes (e.g., MART-1/ Melan-A, gp100, tyrosinase) and 3) unique, mutated antigens (e.g.,  $\beta$ -catenin, CDK4, MUM-1) (reviewed by Van den Eynde and Brichard, 1995).

[0004] It was an object of the present invention to identify novel tumor-associated antigens.

[0005] To solve the problem underlying the invention, melanoma cell line 518A2 and its IL-2- or GM-CSF-transfectants were compared for their CTL stimulating capacity *in vitro*. Stimulation of autologous PBMC with the IL-2 producing melanoma cells resulted in a melanoma-specific CTL response (van Elsas et al., 1997). CTL clones derived from this culture recognized, besides autologous melanoma cell lines, also a panel of HLA-A\*0201 positive melanoma cell lines, but were not reactive with normal melanocytes. Although 518A2 was shown to express a number of antigens previously identified to be recognized by anti-melanoma CTL (van Elsas et al., 1996), the CTL clones available recognize a new melanoma-specific antigen that is immunodominant in 518A2.

[0006] In the experiments of the present invention, the target structure that was recognized by one of these CTL clones was fully characterized and named CAMEL (CTL-recognized Antigen on Melanoma). These sequences are described in the attached sequence listing as SEQ ID NO: 1 and SEQ ID NO: 2. Although the identified CAMEL DNA sequence has high homology and is partially identical to NY-ESO-1, a gene originally identified by SEREX technology (Chen et al., 1997, SEQ ID NO: 7), it was surprisingly found that the CTL epitope of CAMEL is encoded by a reading frame (ORF-1) distinct from that encoding the putative LAGE-1 protein (SEQ ID NO: 4) or NY-ESO-1 protein (SEQ ID NO: 8). LAGE-1 is a gene that has recently been identified by Lethé et al., 1998.

[0007] In the present invention, a cDNA clone was identified that lacks the first 86 bp of the LAGE-1<sup>L</sup> sequence (SEQ ID NO: 5) which means that it is devoid of the initiation codon at position 54 of that sequence (Fig. 2a). The first possible translation initiation site in this clone (4H8) is the ATG at position 94 of LAGE-1<sup>S</sup> (SEQ ID NO: 3), which is however, not in frame with the first ATG at position 54. Therefore, the CAMEL protein (SEQ ID NO: 2) translated from the 4H8 cDNA clone is different from the putative LAGE-1<sup>S</sup> protein (SEQ ID NO: 4).

[0008] In a first aspect, the present invention is directed to tumor-associated antigens encoded by the ORF-1 of LAGE-1 cDNA and by the ORF-1 of cDNAs hybridizing with LAGE-1.

[0009] In the present invention „ORF-1“ is defined as the open reading frame starting with ATG at position 94 of SEQ ID NO:3 (LAGE-1<sup>S</sup>), which corresponds to position 10 in SEQ ID NO: 1 (CAMEL), to position 96 in SEQ ID NO: 5 (LAGE-1<sup>L</sup>) and to position 94 of SEQ ID NO: 7 (NY-ESO-1).

[0010] In an embodiment of the invention, the antigen is CAMEL (SEQ ID NO: 2), which is encoded by the ORF-1 of the LAGE-1 cDNA.

[0011] In another embodiment, the invention is directed to a polypeptide encoded by the ORF-1 of the NY-ESO-1 cDNA (SEQ ID NO: 7).

[0012] Additional members of a gene family including LAGE-1 and NY-ESO1 can be identified by screening cDNA or genomic DNA libraries from cell lines, e.g. cell lines derived from tumors, or from primary tissues, e.g. tumors, testis, placenta, etc. with a probe comprising the ORF-1 of LAGE-1 or NY-ESO-1, at low stringency conditions, and confirming the existence of an open reading frame corresponding to ORF-1 of LAGE-1. An example for low stringency conditions is hybridization at 60°C and washing at 2XSSC at 60°C, or equivalent conditions in Church buffer or SSCP, as described in standard protocols, e.g. Sambrook et al., 1989.

[0013] An alternative method that may be used to identify LAGE-1 family members with ORF-1, is Representational Difference Analysis. This PCR-based method has been proven useful to identify genes with tissue-specific or tumor-specific expression (Lethé et al., 1998). By means of this method, LAGE-1 and NY-ESO1 were identified by screening cDNA libraries from melanoma cell lines with a primer from a cDNA clone enriched in melanoma-specific sequences.

[0014] In a further aspect, the present invention relates to immunogenic (poly)peptides derived from the tumor-associated antigens of the invention. A first group of peptides is selected from peptides inducing a humoral immune response (induction of antibodies). Such peptides are selected by randomly choosing continuous stretches of amino acids (at least 12-15), applying them to an individual and confirming the generation of antibodies by standard immunological assays, e.g. ELISA. This group of immunogenic (poly)peptides also encompasses the entire antigen or larger fragments thereof.

[0015] The second group of peptides, which is preferred, can be presented by MHC molecules (in humans: HLA molecules), they have the potential to induce an immune response, in particular by eliciting a CTL response.

[0016] In a preferred embodiment, the immunogenic peptides are derived from CAMEL.

[0017] In a preferred embodiment, immunogenic peptides which have the ability to elicit a CTL response, are selected from peptides with the sequence Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu (SEQ ID NO: 11) or Leu Met Ala Gln Glu Ala Leu Ala Phe Leu (SEQ ID NO: 12)

[0018] To obtain peptides that have the ability to elicit a cellular immune response, the selection of peptide sequences from a given antigen is, in the first place, based on the requirement for such peptide to bind to an MHC molecule present in the repertoire of the patient to be treated. Two classes of MHC molecules are distinguished, class I and class II. Class I molecules consist of a membrane-inserted heavy chain and a non-covalently attached light chain. In their structure, MHC class I molecules resemble a moose's head, the antlers forming a groove which is recognized by the peptide. In humans, HLA-A, B and C are the "classical" MHC class I molecules.

[0019] Additional immunogenic peptides may be identified by methods known in the art which rely on the correlation between MHC-binding and CTL induction, e.g. those used by Stauss et al., 1992, who identified candidate T-cell epitopes in human papilloma virus.

[0020] Since immunogenic peptides can be predicted based on their "peptide binding motif" synthetic peptides which represent CTL epitopes may be designed and synthesized. Several methods, which are useful in the present invention for designing peptides, have been used to identify CTL epitopes from known protein antigens.

[0021] It is well established that every MHC class I allelic product has allele-specific requirements for the peptide ligand that binds to its groove and that it ultimately presents. These requirements were summarized as a motif by Falk et al., 1991. A large number of MHC peptide motifs and MHC ligands have become known to date. A method to search a known protein sequence for epitopes fitting to a given class I molecule, which is based on this knowledge and which may be used in the present invention, was reviewed by Rammensee et al., 1995. It comprises the following steps: first, the protein sequence is screened for stretches fitting to the basic anchor motif (two anchors in most cases), whereby allowance should be made for some variation in peptide lengths as well as in anchor occupancy. If a motif, for example calls for 9mers with Ile or Leu at the end, 10mers with a fitting C-terminus should be considered as well, and other aliphatic residues at the C-terminus, like Val or Met, should also be considered. In this way, a list of peptide candidates is obtained. These are inspected for having as many non-anchor residues as possible in common with ligand already known, or with the residues listed among the "preferred residues" or "others" on top of each motif (Table, given by Rammensee et al., 1995), for various HLA molecules. Binding assays can be performed at this stage to exclude weak binders which occur frequently among peptides conforming to a basic motif. If a detailed study on peptide binding requirements is available, the candidates can also be screened for non-anchor residues detrimental or optimal for binding (Ruppert et al., 1993). One should keep in mind, however, that pure peptide binding motifs can be misleading in the search for natural ligands, since other constraints, such as enzyme specificity during antigen processing and specificity of transporters or chaperones, are likely to contribute to ligand identity in addition to the MHC binding specificity.

[0022] This approach was successfully applied by, inter alia, Kawakami et al., 1995, to identify gp100 epitopes based on known HLA-A2.1 motifs. The validity of the method was confirmed by identifying, in parallel, the epitope regions by using COS cells transfected with cDNA fragments generated by sequential deletion and testing for T-cell reactivity, as described above.

[0023] Recently, data bases and prediction algorithms have become available that enable to predict, with great reliability, peptide epitopes that bind to HLA molecules of interest.

[0024] Examples for peptide candidates with potential immunogenicity that can be derived from the tumor-associated antigens of the present invention, are the CAMEL-derived peptides with the sequence HLSPDQGRE and LMAQEALAF for HLA-A3 or RMAVPLLR for HLA-A3101. Similarly, other peptides for these or for other alleles can be determined by the method mentioned above.

[0025] The peptide binding can be tested in peptide binding assays. In order to determine the immunogenicity of the selected peptide or peptide equivalent, as defined below, which is the crucial parameter for peptide-based vaccine development and which in most cases strongly correlates with the stability of the peptide-MHC interaction (van der Burg et al, 1996), the methods described by Sette et al., 1994, in combination with quantitative HLA-binding assays, may be used. Alternatively, immunogenicity of the selected peptide may be checked by performing *in vitro* CTL induction by known methods e.g. as described below for *ex vivo* CTL induction.

[0026] Alternatively to peptides derived from the naturally expressed tumor antigens, functional equivalents thereof, i.e. peptides with partially altered sequences or substances mimicking peptides, e.g. "peptidomimetics" or retro-inverso peptides, may be obtained by the following methods:

[0027] To enhance the immunogenicity of the peptides, amino acid substitutions may be introduced at anchor positions to increase peptide MHC class I-binding affinity. The modified peptides are subsequently evaluated for enhanced binding and immunogenicity by screening for recognition by TIL (tumor-infiltrating lymphocytes) and CTL induction as described by Parkhurst et al, 1996, and Bakker et al., 1997.

[0028] Another method useful in the present invention to find more immunogenic peptides by screening peptide libraries with a known CTL was described by Blake et al. 1996; it suggests the use of combinatorial peptide libraries for constructing functional mimics of tumor epitopes recognized by MHC class I-restricted CTLs.

5 [0029] In principle, the selection of peptides capable of eliciting a cellular immune response is carried out in several steps, as described in WO 97/30721, which disclosure is incorporated herein by reference. In short, the candidates are first tested for their binding ability to an MHC molecule; subsequently good binders are tested for immunogenicity. A general strategy for obtaining efficient immunogenic peptides has been described by Schweighoffer, 1997.

[0030] The polypeptides of the present invention or immunogenic peptides derived from their sequence, respectively, can be produced recombinantly or by peptide synthesis, as described in WO 96/10413, the disclosure of which 10 is incorporated herein by reference. For recombinant production, a DNA molecule encoding the antigen or the CTL peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell, cultivated under conditions suitable for expression, recovered and purified. For chemical synthesis, various conventional techniques may be used, e.g. commercially available automatic synthesizers.

[0031] The tumor antigens of the present invention and the immunogenic peptides derived therefrom or the respective peptide equivalents are useful in cancer therapy, e.g. to induce, in the context of the appropriate MHC presenting 15 molecule, an immunological response to tumors which express the corresponding antigen determinants. The induction of CTLs can be accomplished *in vivo* or *ex vivo*.

[0032] For *in vivo* induction of CTLs, a pharmaceutical composition comprising the peptide/antigen is administered to an individual suffering from a tumor associated with the respective tumor antigen in an amount sufficient to elicit an 20 effective CTL response to the antigen-bearing tumor. Thus, the present invention provides pharmaceutical compositions for therapeutic treatment which are intended for parenteral, topical, oral or local administration. Preferably, the compositions are for parenteral administration, e.g. for intravenous, subcutaneous, intradermal or intramuscular application. The peptides/antigens are dissolved or suspended in a pharmaceutically acceptable carrier, preferably an aqueous carrier. The composition may contain additional auxiliary substances, e.g. buffering agents, etc. The peptides may 25 be used alone or in combination with adjuvants, e.g. saponins, albumin, or, in a particularly preferred embodiment, polycations, like polyarginine or polylysine. The peptides may also be linked to components assisting CTL priming, e.g. T helper peptides, lipids or liposomes or coadministered with such components or with immunostimulating substances, e.g. cytokines (IL-2, IFN- $\gamma$ ). Methods and compositions for preparing and administering pharmaceutical compositions for therapeutic treatments are described in WO 95/04542 and WO 97/30721 the disclosures of which are herein incorporated by reference. 30

[0033] The immunogenic peptides may also be used to elicit a CTL response *ex vivo*. An *ex vivo* CTL response to a tumor expressing the antigen is induced by incubating a patient's CTL precursor cells together with antigen presenting cells and the immunogenic peptide. The thus activated CTLs are allowed to mature and expand to effector CTLs which are then readministered to the patient. Alternatively, the tumor antigen may be pulsed onto APCs which present MHC 35 class II-reactive peptides (Mayordomo et al., 1995; Zitvogel et al., 1996). A suitable method for loading peptides onto cells, e.g. dendritic cells, is disclosed in WO 97/19169.

[0034] The peptides of the invention are preferably applied as a combination of peptides, e.g. different peptides from one or more antigens of the present invention. In an even more preferred embodiment, the peptides of the invention are combined with peptides derived from other tumor antigens. The selection of the peptides is optimized towards 40 covering multiple HLA types in order to be useful for a broad population of patients and/or towards a broad variety of malignancies, which is taken into account by combining peptides from a large variety of tumor antigens. The number of peptides suitable to be combined to yield an efficient therapy may vary within a broad range, e.g. from about 2 to approximately 100.

[0035] In a further aspect, the present invention is directed to isolated DNA molecules comprising ORF-1 of LAGE-1 cDNA and the ORF-1 of cDNAs hybridizing with LAGE-1 under low stringency conditions. 45

[0036] In a further aspect, the invention relates to an isolated cDNA molecule encoding CAMEL.

[0037] In a preferred embodiment, the DNA molecule encoding CAMEL comprises nucleotides 54 - 336 of the sequence set forth in SEQ ID NO: 1.

[0038] In a further aspect, the invention relates to an isolated DNA molecule comprising ORF-1 of the NY-ESO-1 cDNA (the sequence of NY-ESO-1 is depicted in SEQ ID NO: 9 and 10 for cDNA and protein respectively). 50

[0039] The DNAs of the present invention, or the corresponding RNAs, may be used, as an alternative to the use of the protein or the peptide, for cancer immunotherapy. Alternatively to using the natural sequence or fragments thereof, engineered derivatives may be utilized. These include sequences modified to encode (poly)peptides with improved immunogenicity, e.g. taking into account the modifications described above for the peptides. Another form of 55 modification is the assembly of multiple sequences encoding immunologically relevant peptides in a so-called string-of-beads fashion, as described by Toes et al., 1997. The sequences may also be modified by adding auxiliary coding elements, e.g. targeting functions that ensure more efficient delivery and processing of the immunogen (e.g. Wu et al., 1995).

[0040] The nucleic acid molecules may be delivered either directly or as part of a recombinant virus or bacterium. Recombinant in principle, any method that is known for gene therapy may be applied for nucleic acid-based cancer immunotherapy, both *in vivo* and *ex vivo*.

[0041] Examples for *in vivo* delivery are direct injection (injection of "naked" DNA) either intramuscularly or by "gene gun", which has been shown to result in the generation of CTLs against tumor antigens. Examples for recombinant organisms are vaccinia virus, fowlpox virus and adenovirus or *Listeria monocytogenes* (see Coulie, 1997 for a comprehensive review). Furthermore, synthetic nucleic acid carriers like cationic lipids, microspheres, microbeads, liposomes may be useful for *in vivo* delivery of the sequence encoding respective antigen/peptide. Similarly as for peptides, various auxiliary agents that enhance the immune response may be co-applied, e.g. cytokines, either as proteins or as plasmids encoding these.

[0042] Examples for *ex vivo* delivery are transfection of dendritic cells (Tuting, T., 1997) or other antigen presenting cells which are applied as a cellular cancer vaccine.

[0043] The present invention is also directed to the use of cells that express the tumor-associated antigens of the invention, either naturally or upon transfection with the respective coding sequence, for the preparation of a tumor vaccine.

[0044] In the present invention, it has been shown that CTL clones raised against IL-2 producing melanoma cell line 518/IL-2.14 are reactive against two alternatively spliced variants of LAGE-1, LAGE-1<sup>S</sup> (SEQ ID NO: 3) and LAGE-1<sup>L</sup> (SEQ ID NO: 5) and NY-ESO-1 (SEQ ID NO: 9). NY-ESO-1 is a recently described tumor antigen, identified by screening a cDNA library of an esophagus carcinoma with autologous patient serum (SEREX-method (Chen et al., 1997)). NY-ESO-1 is expressed in different tumor types but not in healthy tissues except the testis.

[0045] In the present invention, the epitope of specific CTL 1/29 was determined by cDNA expression cloning and a truncated LAGE-1 cDNA clone was found. This truncation led to the identification of the peptide epitope in an alternative reading frame, since the "normal" translation initiation site of LAGE-1 was absent. However, COS/HLA-A\*0201 cells transfected with full length LAGE-1 or NY-ESO-1 cDNA clones could stimulate the CTL clone to TNF production as well. This probably means that two different proteins can be translated from one single mRNA.

[0046] NY-ESO-1 also has been described as the target of melanoma-specific HLA-A\*0201 restricted CTL clones, which recognize a an epitope translated in ORF3, located between aa 155 and 167 (Jager et al., 1998). Therefore, it is very likely that also LAGE-1<sup>S</sup> will be recognized by these clones, but not LAGE-1<sup>L</sup>, since the protein sequence is different at this part of the molecule. Our CTL clones recognize a peptide in an alternative reading frame, which is encoded in both LAGE-1 and NY-ESO-1. This means that tumor cells expressing either LAGE-1 or NY-ESO-1 can be recognized by MLMAQEALAFI-specific CTL, which might enlarge the number of tumors that can be treated with immunotherapy based on this peptide.

Brief description of the Figures:

[0047]

Figure 1: COS-7 transfection experiments with cDNA clone CAMEL and deletion constructs

a) COS-7 cells were transfected with cDNAs as indicated and tested with CTL 1/29 in a TNF release assay.

b) Deletion constructs of CAMEL cDNA were cotransfected with HLA-A\*0201 cDNA in COS-7, followed by a TNF release assay with CTL 1/29. The PCR clones contain the numbers of nucleotides of the CAMEL cDNA as indicated.

Figure 2:

a) Nucleotide alignment of cDNA clones CAMEL, LAGE-1<sup>S</sup>, LAGE-1<sup>L</sup> and NY-ESO-1.

b) Protein translations of the cDNA clones LAGE-1<sup>S</sup>, LAGE-1<sup>L</sup> and NY-ESO-1. The translation of CAMEL is identical to the translation of LAGE-1<sup>SL</sup> in ORF1. Although ORF3 seems the most putative one, the CTL epitope is encoded in ORF1 (underlined).

Figure 3: Characterisation of peptides recognized by CTL clone 1/29

a) TNF release assay with predicted HLA-A\*0201 binding CAMEL peptides. Peptides as indicated were loaded on BLM, an HLA-A\*0201<sup>+</sup> melanoma cell line, at a concentration of 10 µg/ml and tested

with CTL 1/29 in a TNF release assay.

b) The effects of increasing concentrations of peptides, derived from the major target epitope MLMAQEALAFI on recognition by CTL 1/29. Various concentrations of peptides as indicated were loaded on HLA-A\*0201\* cells and tested in a TNF release assay with CTL 1/29.

**Figure 4:** LAGE-1<sup>S/L</sup> (and NY-ESO-1) both encode the CTL epitope

COS/HLA-A\*0201 cells were transfected with these cDNA clones and reactivity with CTL 1/29 was measured in a TNF release assay.

**Figure 5:** His-tagged CAMEL protein, synthesized in E.coli

**Figure 6:** Expression of LAGE-1<sup>S/L</sup> and NY-ESO-1 in healthy human tissues and melanoma cell lines

a) Northern Blot analysis of the expression of LAGE-1/NY-ESO-1 in a panel of healthy human tissues as indicated. The Blot was hybridised with <sup>32</sup>P-dCTP-labeled LAGE-1<sup>S</sup> cDNA.

b) RT-PCR for LAGE-1 and NY-ESO-1. To discriminate between LAGE-1 and NY-ESO-1 mRNA, the same panel of melanoma cell lines was analysed by RT-PCR with gene-specific primers. Melanoma cell lines as indicated were used as targets in a TNF release assay with CTL 1/29.

Brief description of the sequences:

[0048]

SEQ ID NO: 1: CAMEL (H8) cDNA sequence and translation  
 SEQ ID NO: 2: CAMEL protein sequence  
 SEQ ID NO: 3: LAGE-1<sup>S</sup> cDNA sequence and translation  
 SEQ ID NO: 4: LAGE-1<sup>S</sup> protein sequence  
 SEQ ID NO: 5: LAGE-1<sup>L</sup> cDNA sequence and translation  
 SEQ ID NO: 6: LAGE-1<sup>L</sup> protein sequence  
 SEQ ID NO: 7: NY-ESO-1 cDNA sequence and translation  
 SEQ ID NO: 8: NY-ESO-1 protein sequence  
 SEQ ID NO: 9: NY-ESO-1 cDNA and alternative translation  
 SEQ ID NO: 10: protein sequence of alternatively translated NY-ESO-1  
 SEQ ID NO: 11: peptide sequence of the CAMEL CTL epitope (11-mer)  
 SEQ ID NO: 12: peptide sequence of the CAMEL CTL epitope (10-mer)  
 SEQ ID NO: 13: oligonucleotide SP6F-pSV  
 SEQ ID NO: 14: oligonucleotide R1  
 SEQ ID NO: 15: oligonucleotide R2  
 SEQ ID NO: 16: oligonucleotide T7R-pSV  
 SEQ ID NO: 17: oligonucleotide F3  
 SEQ ID NO: 18: oligonucleotide ESO-1B  
 SEQ ID NO: 19: oligonucleotide ESO-1A  
 SEQ ID NO: 20: oligonucleotide 4H8-A  
 SEQ ID NO: 21: oligonucleotide 4H8-C

[0049] In the Examples, if not stated otherwise, the following materials and methods were used

a) Cell cultures

[0050] Melanoma cell lines and COS-7 cells were maintained in DMEM containing 4.5 mM glucose supplemented with 8% FCS, 2 mM L-glutamine, 100 µg/ml of each penicillin and streptomycin. Melanoma cell line 518A2 was established in our laboratory from the dissected metastasis of a male patient in 1985, as described before (Versteeg et al., 1988). An IL-2 producing variant, 518/IL-2.14, was obtained by transfection of 518A2 with the IL-2 cDNA (Osanto et al., 1993). Other melanoma cells that were used as targets in TNF release assay are FM3.29, FM6, FM28.4 and FM55P (gifts from J. Zeuthen, Denmark), MM127, MM415, MM485 (gifts from N. Hayward, Australia), SK-MEL-23, SK-MEL-29

(obtained from T. Wölfel, Mainz), Mi10221, Mi3046/2, NA8, BLM (obtained from M. Visseren, Leiden). EBV-transformed B-LCL and the TNF-sensitive WEHI-164 clone 13 (a gift from Dr. P. Coulie, Brussels) were cultured in RPMI-1640, supplemented with L-glutamine and antibiotics as above, and 10% FCS.

5 [0051] With the IL-2 producing cell line 518/IL-2.14 and autologous peripheral blood mononuclear cells (PBMC) a CTL induction was performed, resulting in melanoma-specific HLA-A\*0201 restricted CTL clones (van Elsas et al., 1997). The identification of the epitope of one of these clones, CTL 1/29, is reported here.

b) cDNA expression cloning

10 [0052] A cDNA library of 518/IL-2.14 was constructed in the expression vector pSVsport1 (GIBCO, BRL) using the Superscript Plasmid System (GIBCO, BRL). As to that purpose, poly-A<sup>+</sup> mRNA was isolated using the Fast-Track system (Invitrogen), followed by reverse-transcription with an oligo-dT/NotI primer. Sall adapters were ligated to ds-cDNA and after NotI digestion and size fractionation, cDNA fragments were cloned into the pSVsport1 vector digested with Sall and NotI. After electroporation into ElectroMAX-DH10B (GIBCO, BRL) (following the manufacturers instructions) and selection for ampicillin resistance, 50-100 colonies were pooled for mini DNA isolation (QIAprep 8 plasmid kit, Qia-  
15 gen). The in this way obtained cDNA pools were transfected in duplicate into COS-7 cells, together with the restriction element HLA-A\*0201 (pBJ1.neo/HLA-A\*0201, (Lin et al., 1990)), using the DEAE-dextran method. Briefly, COS-7 cells were seeded in 96-wells flatbottom plates at  $1.5 \times 10^4$  cells per well in 100  $\mu$ l DMEM, 8% FCS. After 2 hours, medium was replaced by 30  $\mu$ l transfection mixture, containing 100 ng cDNA pool, 100 ng HLA-A\*0201 cDNA, 400 ng/ml DEAE-dextran and 100  $\mu$ M chloroquine in serum free DMEM. Cells were incubated for 4 hours at 37°C and shocked for 2 minutes by the addition of 50  $\mu$ l 10% DMSO in PBS. The shock medium was replaced by 200  $\mu$ l DMEM, 8% FCS, and 48 hours later the cells were used as target cells for CTL in a TNF release assay.

c) Deletion constructs

25 [0053] Deletion constructs of cDNA clone 4H8 were obtained by PCR. PCR products were cloned in vector pCR3.uni (TA cloning system, Invitrogen). The constructs pCR-246 and pCR-464 were made with the vector-based forward primer, SP6F-pSV (SEQ ID NO: 13) and the reverse primers in cDNA 4H8, R1 (SEQ ID NO: 14) and R2 (SEQ ID NO: 15) respectively. As a control the complete 679 bp cDNA was cloned by PCR with two primers on the pSVsport  
30 vector, SP6F-pSV (SEQ ID NO: 13) and T7R-pSV (SEQ ID NO: 16), resulting in pCR-679.

d) TNF release assay

[0054] CTL reactivity against tumor target cells, transfected COS-7 or peptide loaded cells was measured in a TNF  
35 release assay. Target cells were seeded in duplicate or triplicate at  $1.5 \times 10^4$  cells per well in a 96-wells flat bottom plate and 1500-2000 CTL were added to each well, in a total volume of 100  $\mu$ l / well (IMDM, supplemented with antibiotics and 5% FCS). After 24 hours of co-culturing of effector and target cells, 50  $\mu$ l out of each well was added to a fresh 96-wells flatbottom plate, containing 50  $\mu$ l ( $4.5 \times 10^4$ ) TNF-sensitive WEHI-164 cells per well in IMDM, supplemented with antibiotics, 5% FCS, 2  $\mu$ g/ml Actinomycin D and 40 mM LiCl. A viability staining was performed 24 hours later by  
40 the addition of 50  $\mu$ l of 3-(4,5dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) solution (2.5 mg/ml in PBS). After incubation for 2-4 hours at 37°C the OD<sub>550-650</sub> was measured. TNF release in pg/ml was calculated from a standard with known TNF concentrations.

e) Northern Blot analysis

45 [0055] To determine expression in healthy tissues a Multiple Tissue Northern Blot was obtained commercially (Clontech). As a probe, LAGE-1 cDNA was used, labeled with  $\gamma$ -<sup>32</sup>P-dCTP by use of the Mega-Prime Labeling kit (Amersham).

50 f) RT-PCR

[0056] cDNA synthesis was performed using oligo-dT and M-MLV reverse transcriptase (Promega). Primers used for LAGE-1 specific PCR were the F3 (SEQ ID NO: 17) and ESO-1B primer (SEQ ID NO: 18). ESO-1B was also used as a reverse primer in the NY-ESO-1-specific PCR, while ESO-1A (SEQ ID NO: 19) was the forward primer in this reac-  
55 tion (Chen et al., 1997). Reactions were performed in a Biometra-Uno or -Trio programmed as follows: 5 minutes 95°C, 30 cycles of 1 min. 95°C, 1 min. 58°C, 1 min. 72°C, followed by 10 minutes 72°C.

g) Expression of CAMEL in E. Coli

[0057] A fragment containing the coding sequence of CAMEL was made by PCR with the following primers:

5 4H8-A: GAAGAACATATGCTGATGGCCCAGGAGGC (SEQ ID NO: 20)  
4H8-C: TTAAAGATCTCAGAACCGCCCCTGGTCG (SEQ ID NO: 21)

[0058] This fragment was digested with NdeI and BglII and cloned in the NdeI and BamHI sites of vector pET19b (Novagen, Madison, WI). This vector contains a 6xHis-tag coding sequence, allowing detection of the His-tagged protein with an anti-His antibody. The pET19b-CAMEL construct was transformed into BL21(DE3)pLysS E. coli bacteria (Novagen, Madison, WI). After culturing the bacteria at 30°C until an OD<sub>600</sub> = 0.5, IPTG (1 mM) was added to induce overexpression of the His-tagged CAMEL protein. Samples were taken at 0h and 4h after IPTG induction and lysates of these samples were tested on a Western Blot with the Penta-His Antibody (Qiagen) according to the Western and Colony Blot protocol of the supplier. The His-tagged protein was visualized using the SuperSignal Substrate system for Western blotting (Pierce, Rockford, US).

Example 1

cDNA clone 4H8 (CAMEL) encodes the target for melanoma-specific CTL1/29

20 [0059] The antigenic epitope of melanoma-specific CTL 1/29 was identified by the expression of cDNA library 518/IL2.14 and the restriction element HLA-A\*0201 in COS-7 cells, followed by CTL screening in a TNF release assay. A positive pool of cDNAs was subcloned and clone 4H8, called CAMEL (SEQ ID NO: 1), was found to stimulate TNF release by the CTL to a similar extent as the original 518/IL2.14 cell line (Fig. 1). COS-7 cells or COS-7 cells transfected with HLA-A\*0201 or the 4H8 cDNA only were not recognized. The isolated 4H8 cDNA clone has a 679 bp insert, which shows strong homology with NY-ESO-1 (SEQ ID NO: 7), a tumor antigen originally identified as a target for humoral immune responses by serum screening methods (SEREX) (Chen et al., 1997). Colony hybridization of the cDNA library, using clone 4H8 as a probe resulted in the detection of 2 types of full length clones which we call LAGE-1<sup>S</sup> (SEQ ID NO: 3) and LAGE-1<sup>L</sup> (SEQ ID NO: 5) (Fig. 2a). LAGE-1<sup>L</sup> contains a 229 bp insertion at position 457, which has the consensus sequences for an intron, starting with a 5' GT and ending 3' AG. This indicates alternative splicing of LAGE-1 mRNA. However, cDNA clone 4H8 lacks the first 84 bp of the LAGE-1 cDNA sequence.

Example 2

35 The peptide epitope of CTL 1/29 is coded in an alternative reading frame of LAGE-1 or NY-ESO-1

[0060] To identify which peptide was recognized by CTL 1/29, deletion constructs of cDNA 4H8 were transfected in HLA-A\*0201<sup>+</sup> COS-7 cells and tested in a TNF release assay. CTL reactivity was measured with all constructs (Fig. 1b), indicating that the epitope was coded within the first 330 bp of clone 4H8. An HLA-A\*0201 binding motif search was performed on the predicted protein sequence of that region (Drijfhout et al., 1995; D'Amato et al., 1995), presuming that the ATG at position 10 in 4H8 functions as the translation initiation site. Predicted strong binding peptides at regions 1-11, 2-11, 1-9, 10-18, 11-19, 16-25, 17-25, 49-57, 55-63 and 70-78 of the CAMEL protein sequence (Fig. 2b) were added to HLA-A\*0201<sup>+</sup> BLM melanoma cells, and tested for CTL reactivity in a TNF release assay (Fig. 3b).

45 [0061] At a peptide concentration of 10 µg/ml only the N-terminal 11- and 10-mer peptides (M) LMAQEALAF (SEQ ID NO: 11 and NO: 12) induced preponderant recognition by CTL 1/29 (Fig. 3a), indicating that the epitope recognized by the CTL is located in the first 11 amino acids of the CAMEL-encoded protein. Closer inspection of peptides derived of this N-terminal 11-mer in a peptide concentration dependent TNF release assay (Fig. 3b) revealed that the methionine at position 1 as well as the leucine at position 11 are of crucial importance for reconstituting CTL reactivity. Deletion of either of these amino acids leads to an at least 5 decades higher peptide concentration required for comparable TNF release. The only other peptide showing weak activity is the 3-11 MAQEALAF. In contrast, the MLMAQEALA has no activity at all (Fig. 3b), suggesting that the C-terminal amino acids FL do significantly contribute to the recognition by the CTL.

Example 3

55 Comparison of CAMEL, LAGE-1<sup>S/L</sup>, NY-ESO-1

[0062] As already mentioned, cDNA clone 4H8 lacks the first 84 bp of the LAGE-1 sequence, which means that it



is devoid of the initiation codon at position 54 of that sequence (Fig. 2a). The first possible translation initiation site in 4H8 corresponds with the ATG at position 94 of LAGE-1, which is however, not in frame with the first ATG at position 54. Therefore, the protein translated from the 4H8 cDNA clone is different from the putative LAGE-1 protein, since translation takes place in another reading frame (Fig. 2a and b). 4H8 encodes a protein of 109 amino acids (SEQ ID NO: 2) with a predicted molecular weight of 11.7 kD. The LAGE-1<sup>S</sup> protein translated from the first ATG will be a 180 aa protein of 18.2 kD (SEQ ID NO: 4), while the unspliced variant, LAGE-1<sup>L</sup>, encodes a 210 aa protein of 21.1 kD (SEQ ID NO: 6). NY-ESO-1 protein (SEQ ID NO: 8) is probably of the same size as LAGE-1<sup>S</sup>, but differs at 26 amino acids. However, if translation of LAGE-1<sup>S/L</sup> starts at the second ATG, proteins will be translated in another reading frame and are in that case identical to the protein translated from cDNA 4H8. Alternative translation of NY-ESO-1 (SEQ ID NO: 9 and NO: 10) results in a shorter variant of this protein (58 amino acids), because of an earlier stop codon (Fig. 2b), which differs from the CAMEL protein sequence only in its last 5 amino acids (Fig. 2b).

**[0063]** It was examined whether cells transfected with the complete LAGE-1 (or NY-ESO-1) cDNA clones are able to stimulate CTL 1/29. Remarkably, COS/HLA-A\*0201 cells transfected with LAGE-1<sup>S</sup>, the alternatively spliced LAGE-1<sup>L</sup>, (as well as with the NY-ESO-1) cDNA are able to stimulate CTL 1/29 (Fig. 4). This indicates that, at least in COS-7 cells, protein translation also starts from the second start codon at nucleotide 94 in LAGE-1<sup>S</sup>, notwithstanding the presence of the first ATG at position 54. Also in this case, this results in the "alternative reading frame" peptide, MLMAQEALAF<sub>L</sub>, recognized by CTL 1/29.

#### Example 4

##### Expression of CAMEL in E. Coli

**[0064]** To investigate whether CAMEL is indeed translated from the ORF-1 of the CAMEL (4H8) cDNA, the CAMEL cDNA (SEQ ID No: 1) was cloned in a bacterial expression vector (pET19b) (Studier et al., 1990). This vector contains a 6xHis-tag coding sequence, allowing detection of the His-tagged protein with an anti-His antibody. The pET19b-CAMEL construct was transformed into E.coli and the bacteria were treated with IPTG to induce expression of the His-tagged CAMEL protein. Extracts were analyzed by Western blotting using the Penta-His antibody. Western blotting of a lysate shows a 15.5 kD protein, only slightly higher than the expected 14.5 kD of the His-tagged CAMEL protein after staining with a anti-His antibody (Fig. 5).

**[0065]** The CAMEL cDNA (SEQ ID No: 1) was cloned in pET19b and expressed in E.Coli. Lanes 1 and 2 represent the samples taken at 0h, lanes 3 and 4 at 4h after induction with IPTG. Because CAMEL might be an unstable protein, induction of protein expression was performed in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of PMSF (a protease inhibitor). At the left the positions of the molecular weight marker proteins are indicated.

#### Example 5

##### Expression of LAGE-1 and NY-ESO-1 in healthy human tissues and melanoma cell lines

**[0066]** Hybridisation of Multiple Tissue Northern blots containing RNA of healthy human tissues with the LAGE-1<sup>S</sup> cDNA showed high expression in testis and placenta and low, (but clear) expression in heart, skeletal muscle and pancreas (Fig. 6a). The positive signals exist of two bands, probably reflecting LAGE-1<sup>S</sup>/NY-ESO-1 (750 bp) and LAGE-1<sup>L</sup> (1000 bp).

**[0067]** Several melanoma cell lines were tested for expression of LAGE-1 and NY-ESO-1 by (Northern Blot analysis and) RT-PCR (Fig. 6b). Because of the strong homology between both genes, it is not possible to discriminate between LAGE-1 and NY-ESO-1 on Northern Blot. Therefore RT-PCR was performed with specific primers. We found in most cell lines a correlated expression of LAGE-1 and NY-ESO-1; only cell line FM3.29 had expression of LAGE-1, but was negative for NY-ESO-1. Other cell lines expressed either both or none of the two genes (Fig. 6b). There was a good correlation between the level of expression and the recognition by CTL 1/29 (Fig. 6b).

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SEQUENCE LISTING

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(1) GENERAL INFORMATION:

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(i) APPLICANT:

(A) NAME: Boehringer Ingelheim International GmbH  
(B) STREET: Binger Strasse 173  
(C) CITY: Ingelheim am Rhein  
(E) COUNTRY: Germany  
(F) POSTAL CODE (ZIP): 55216  
(G) TELEPHONE: 06132/772282  
(H) TELEFAX: 06132/774377

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(ii) TITLE OF INVENTION: Tumor-associated antigens

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(iii) NUMBER OF SEQUENCES: 21

25

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

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(2) INFORMATION FOR SEQ ID NO: 1:

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(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

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(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapiens  
(F) TISSUE TYPE: Melanoma

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(A) NAME/KEY: 3'UTR  
(B) LOCATION: 340..679

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 (A) NAME/KEY: 5'UTR  
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(A) LENGTH: 109 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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20             50             55             60
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25             65             70             75             80
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(A) LENGTH: 767 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapiens

(F) TISSUE TYPE: Melanoma

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 54..596

EP 1 001 022 A1

(ix) FEATURE:  
 (A) NAME/KEY: 3'UTR  
 (B) LOCATION:597..767

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(ix) FEATURE:  
 (A) NAME/KEY: 5'UTR  
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	Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly	
	20 25 30	
25	CCA GGA GAG GCG GGT GCC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGG	200
	Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly	
	35 40 45	
30	GCA GCA AGG GCC TCG GGG CCG AGA GGA GGC GCC CCG CGG GGT CCG CAT	248
	Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His	
	50 55 60 65	
35	GGC GGT GCC GCT TCT GCG CAG GAT GGA AGG TGC CCC TGC GGG GCC AGG	296
	Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg	
	70 75 80	
40	AGG CCG GAC AGC CGC CTG CTT CAG TTG CAC ATC ACG ATG CCT TTC TCG	344
	Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe Ser	
	85 90 95	
45	TCG CCC ATG GAA GCG GAG CTG GTC CGC AGG ATC CTG TCC CGG GAT GCC	392
	Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala	
	100 105 110	
50	GCA CCT CTC CCC CGA CCA GGG GCG GTT CTG AAG GAC TTC ACC GTG TCC	440
	Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser	
	115 120 125	
55	GGC AAC CTA CTG TTT ATC CGA CTG ACT GCT GCA GAC CAC CGC CAA CTG	488
	Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln Leu	
	130 135 140 145	
60	CAG CTC TCC ATC AGC TCC TGT CTC CAG CAG CTT TCC CTG TTG ATG TGG	536
	Gln Leu Ser Ile Ser Cys Leu Gln Gln Leu Ser Leu Leu Met Trp	
	150 155 160	
65	ATC ACG CAG TGC TTT CTG CCC GTG TTT TTG GCT CAG GCT CCC TCA GGG	584
	Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser Gly	

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165 170 175

5 CAG AGG CGC TAA GCCCAGCCTG GCGCCCCTTC CTAGGTCATG CCTCCTCCCC 636  
Gln Arg Arg \*  
180

10 TAGGGAATGG TCCCAGCACG AGTGGCCAGT TCATTGTGGG GGCCTGATTG TTTGTCGCTG 696  
GAGGAGGACG GCTTACATGT TTGTTTCTGT AGAAAATAAA GCTGAGCTAC GAAAAAAAAA 756  
AAAAAAAAA A 767

15 (2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 180 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

25 Met Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp  
1 5 10 15

Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly  
20 25 30

30 Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala  
35 40 45

Gly Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro  
50 55 60

35 His Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala  
65 70 75 80

Arg Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe  
85 90 95

40 Ser Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp  
100 105 110

Ala Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val  
115 120 125

45 Ser Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln  
130 135 140

50 Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met  
145 150 155 160

Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser

55



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165 170 175

Gly Gln Arg Arg \*

5 180

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 993 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapiens

(F) TISSUE TYPE: Melanoma

(ix) FEATURE:

(A) NAME/KEY: 5'UTR

(B) LOCATION:1..55

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:56..688

(ix) FEATURE:

(A) NAME/KEY: 3'UTR

(B) LOCATION:689..993

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GCATCCTCGT GGGCCCTGAC CTTCTCTCTG AGAGCCGGGC AGAGGCTCCG GAGCC ATG 58

Met

1

CAG GCC GAA GGC CAG GGC ACA GGG GGT TCG ACG GGC GAT GCT GAT GGC 106

Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly

5 10 15

CCA GGA GGC CCT GGC ATT CCT GAT GGC CCA GGG GGC AAT GCT GGC GGC 154

Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly

20 25 30

CCA GGA GAG GCG GGT GCC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGC 202

Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly

35 40 45

55

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5	GCA GCA AGG GCC TCG GGG CCG AGA GGA GGC GCC CCG CGG GGT CCG CAT Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His 50 55 60 65	250
10	GGC GGT GCC GCT TCT GCG CAG GAT GGA AGG TGC CCC TGC GGG GCC AGG Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg 70 75 80	298
15	AGG CCG GAC AGC CGC CTG CTT CAG TTG CAC ATC ACG ATG CCT TTC TCG Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe Ser 85 90 95	346
20	TCG CCC ATG GAA GCG GAG CTG GTC CGC AGG ATC CTG TCC CGG GAT GCC Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala 100 105 110	394
25	GCA CCT CTC CCC CGA CCA GGG GCG GTT CTG AAG GAC TTC ACC GTG TCC Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser 115 120 125	442
30	GGC AAC CTA CTG TTT ATG TCA GTT CGG GAC CAG GAC AGG GAA GGC GCT Gly Asn Leu Leu Phe Met Ser Val Arg Asp Gln Asp Arg Glu Gly Ala 130 135 140 145	490
35	GGG CGG ATG AGG GTG GTG GGT TGG GGG CTG GGA TCC GCC TCC CCG GAG Gly Arg Met Arg Val Val Gly Trp Gly Leu Gly Ser Ala Ser Pro Glu 150 155 160	538*
40	GGG CAG AAA GCT AGA GAT CTC AGA ACA CCC AAA CAC AAG GTC TCA GAA Gly Gln Lys Ala Arg Asp Leu Arg Thr Pro Lys His Lys Val Ser Glu 165 170 175	586
45	CAG AGA CCT GGT ACA CCA GGC CCG CCG CCA CCC GAG GGA GCC CAG GGA Gln Arg Pro Gly Thr Pro Gly Pro Pro Pro Pro Glu Gly Ala Gln Gly 180 185 190	634
50	GAT GGG TGC AGA GGT GTC GCC TTT AAT GTG ATG TTC TCT GCC CCT CAC Asp Gly Cys Arg Gly Val Ala Phe Asn Val Met Phe Ser Ala Pro His 195 200 205	682
55	ATT TAG CCGACTGACT GCTGCAGACC ACCGCCAACT GCAGCTCTCC ATCAGCTCCT Ile * 210	738
	GTCTCCAGCA GCTTTCCTG TTGATGTGGA TCACGCAGTG CTTTCTGCCC GTGTTTTTGG	798
	CTCAGGCTCC CTCAGGGCAG AGGCGCTAAG CCCAGCCTGG CGCCCCCTTC TAGGTCATGC	858
	CTCCTCCCCT AGGGAATGGT CCCAGCACGA GTGGCCAGTT CATTGTGGGG GCCTGATTGT	918
	TTGTCGCTGG AGGAGGACGG CTTACATGTT TGTTCCTGTA GAAAATAAAG CTGAGCTACG	978
	AAAAAAAAAA AAAAA	993

## (2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 210 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```

Met  Gln  Ala  Glu  Gly  Gln  Gly  Thr  Gly  Gly  Ser  Thr  Gly  Asp  Ala  Asp
 1          5          10          15

Gly  Pro  Gly  Gly  Pro  Gly  Ile  Pro  Asp  Gly  Pro  Gly  Gly  Asn  Ala  Gly
          20          25          30

Gly  Pro  Gly  Glu  Ala  Gly  Ala  Thr  Gly  Gly  Arg  Gly  Pro  Arg  Gly  Ala
          35          40          45

Gly  Ala  Ala  Arg  Ala  Ser  Gly  Pro  Arg  Gly  Gly  Ala  Pro  Arg  Gly  Pro
 50          55          60

His  Gly  Gly  Ala  Ala  Ser  Ala  Gln  Asp  Gly  Arg  Cys  Pro  Cys  Gly  Ala
25 65          70          75          80

Arg  Arg  Pro  Asp  Ser  Arg  Leu  Leu  Gln  Leu  His  Ile  Thr  Met  Pro  Phe
          85          90          95

Ser  Ser  Pro  Met  Glu  Ala  Glu  Leu  Val  Arg  Arg  Ile  Leu  Ser  Arg  Asp
30          100          105          110

Ala  Ala  Pro  Leu  Pro  Arg  Pro  Gly  Ala  Val  Leu  Lys  Asp  Phe  Thr  Val
          115          120          125

Ser  Gly  Asn  Leu  Leu  Phe  Met  Ser  Val  Arg  Asp  Gln  Asp  Arg  Glu  Gly
35          130          135          140

Ala  Gly  Arg  Met  Arg  Val  Val  Gly  Trp  Gly  Leu  Gly  Ser  Ala  Ser  Pro
40          145          150          155          160

Glu  Gly  Gln  Lys  Ala  Arg  Asp  Leu  Arg  Thr  Pro  Lys  His  Lys  Val  Ser
          165          170          175

Glu  Gln  Arg  Pro  Gly  Thr  Pro  Gly  Pro  Pro  Pro  Glu  Gly  Ala  Gln
45          180          185          190

Gly  Asp  Gly  Cys  Arg  Gly  Val  Ala  Phe  Asn  Val  Met  Phe  Ser  Ala  Pro
          195          200          205

His  Ile  *
      210

```

## (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 752 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Homo sapiens

(ix) FEATURE:  
 (A) NAME/KEY: 5'UTR  
 (B) LOCATION:1..53

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION:54..596

(ix) FEATURE:  
 (A) NAME/KEY: 3'UTR  
 (B) LOCATION:597..752

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

30	ATCCTCGTGG GCCCTGACCT TCTCTCTGAG AGCCGGGCAG AGGCTCCGGA GCC ATG	56
	Met	
	1	
35	CAG GCC GAA GGC CGG GGC ACA GGG GGT TCG ACG GGC GAT GCT GAT GGC	104
	Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly	
	5 10 15	
40	CCA GGA GGC CCT GGC ATT CCT GAT GGC CCA GGG GGC AAT GCT GGC GGC	152
	Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly	
	20 25 30	
	CCA GGA GAG GCG GGT GCC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGG	200
	Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly	
	35 40 45	
45	GCA GCA AGG GCC TCG GGG CCG GGA GGA GGC GCC CCG CGG GGT CCG CAT	248
	Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro His	
	50 55 60 65	
50	GGC GGC GCG GCT TCA GGG CTG AAT GGA TGC TGC AGA TGC GGG GCC AGG	296
	Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala Arg	
	70 75 80	
55	GGG CCG GAG AGC CGC CTG CTT GAG TTC TAC CTC GCC ATG CCT TTC GCG	344

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	Gly	Pro	Glu	Ser	Arg	Leu	Leu	Glu	Phe	Tyr	Leu	Ala	Met	Pro	Phe	Ala	
				85					90					95			
5	ACA	CCC	ATG	GAA	GCA	GAG	CTG	GCC	CGC	AGG	AGC	CTG	GCC	CAG	GAT	GCC	392
	Thr	Pro	Met	Glu	Ala	Glu	Leu	Ala	Arg	Arg	Ser	Leu	Ala	Gln	Asp	Ala	
			100					105					110				
10	CCA	CCG	CTT	CCC	GTG	CCA	GGG	GTG	CTT	CTG	AAG	GAG	TTC	ACT	GTG	TCC	440
	Pro	Pro	Leu	Pro	Val	Pro	Gly	Val	Leu	Leu	Lys	Glu	Phe	Thr	Val	Ser	
			115				120					125					
15	GGC	AAC	ATA	CTG	ACT	ATC	CGA	CTG	ACT	GCT	GCA	GAC	CAC	CGC	CAA	CTG	488
	Gly	Asn	Ile	Leu	Thr	Ile	Arg	Leu	Thr	Ala	Ala	Asp	His	Arg	Gln	Leu	
			130			135				140					145		
20	CAG	CTC	TCC	ATC	AGC	TCC	TGT	CTC	CAG	CAG	CTT	TCC	CTG	TTG	ATG	TGG	536
	Gln	Leu	Ser	Ile	Ser	Ser	Cys	Leu	Gln	Leu	Ser	Leu	Leu	Met	Trp		
				150					155					160			
25	ATC	ACG	CAG	TGC	TTT	CTG	CCC	GTG	TTT	TTG	GCT	CAG	CCT	CCC	TCA	GGG	584
	Ile	Thr	Gln	Cys	Phe	Leu	Pro	Val	Phe	Leu	Ala	Gln	Pro	Pro	Ser	Gly	
				165				170					175				
30	CAG	AGG	CGC	TAA	GCCCAGCCTG	GCGCCCTTC	CTAGGTCATG	CCTCCTCCCC									636
	Gln	Arg	Arg	*													
				180													
35	TAGGGAATGG	TCCCAGCACG	AGTGGCCAGT	TCATTGTGGG	GGCCTGATTG	TTGTGCTGCTG											696
40	GAGGAGGACG	GCTTACATGT	TTGTTTCTGT	AGAAAATAAA	ACTGAGCTAC	GAAAAA											752

(2) INFORMATION FOR SEQ ID NO: 8:

35	(i) SEQUENCE CHARACTERISTICS:															
	(A) LENGTH: 180 amino acids															
	(B) TYPE: amino acid															
	(D) TOPOLOGY: linear															
40	(ii) MOLECULE TYPE: protein															
45	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:															
	Met	Gln	Ala	Glu	Gly	Arg	Gly	Thr	Gly	Gly	Ser	Thr	Gly	Asp	Ala	Asp
	1				5				10					15		
50	Gly	Pro	Gly	Gly	Pro	Gly	Ile	Pro	Asp	Gly	Pro	Gly	Gly	Asn	Ala	Gly
				20				25						30		
55	Gly	Pro	Gly	Glu	Ala	Gly	Ala	Thr	Gly	Gly	Arg	Gly	Pro	Arg	Gly	Ala
			35				40					45				
60	Gly	Ala	Ala	Arg	Ala	Ser	Gly	Pro	Gly	Gly	Gly	Ala	Pro	Arg	Gly	Pro
		50				55						60				

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His Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala  
65 70 75 80

5 Arg Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe  
85 90 95

Ala Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp  
100 105 110

10 Ala Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val  
115 120 125

Ser Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln  
130 135 140

15 Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met  
145 150 155 160

Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser  
165 170 175

20 Gly Gln Arg Arg \*

180

25

(2) INFORMATION FOR SEQ ID NO: 9:

30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 752 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

40 (vi) ORIGINAL SOURCE:  
(A) ORGANISM: homo sapiens

(ix) FEATURE:  
(A) NAME/KEY: 5'UTR  
45 (B) LOCATION:1..93

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION:94..270

50 (ix) FEATURE:  
(A) NAME/KEY: 3'UTR  
(B) LOCATION:271..752

55

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

5	ATCCTCGTGG GCCCTGACCT TCTCTCTGAG AGCCGGGCAG AGGCTCCGGA GCCATGCAGG	60
	CCGAAGGCCG GGGCACAGGG GGTTCGACGG GCG ATG CTG ATG GCC CAG GAG GCC	114
	Met Leu Met Ala Gln Glu Ala	
	1 5	
10	CTG GCA TTC CTG ATG GCC CAG GGG GCA ATG CTG GCG GCC CAG GAG AGG	162
	Leu Ala Phe Leu Met Ala Gln Gly Ala Met Leu Ala Ala Gln Glu Arg	
	10 15 20	
15	CGG GTG CCA CGG GCG GCA GAG GTC CCC GGG GCG CAG GGG CAG CAA GGG	210
	Arg Val Pro Arg Ala Ala Glu Val Pro Gly Ala Gln Gly Gln Gln Gly	
	25 30 35	
20	CCT CGG GGC CGG GAG GAG GCG CCC CGC GGG GTC CGC ATG GCG GCG CGG	258
	Pro Arg Gly Arg Glu Glu Ala Pro Arg Gly Val Arg Met Ala Ala Arg	
	40 45 50 55	
25	CTT CAG GGC TGA ATGGATGCTG CAGATGCGGG GCCAGGGGGC CGGAGAGCCG	310
	Leu Gln Gly *	
30	CCTGCTTGAG TTCTACCTCG CCATGCCTTT CGCGACACCC ATGGAAGCAG AGCTGGCCCG	370
	CAGGAGCCTG GCCCAGGATG CCCACCGCT TCCCGTGCCA GGGGTGCTTC TGAAGGAGTT	430
	CACTGTGTCC GGCAACATAC TGACTATCCG ACTGACTGCT GCAGACCACC GCCAACTGCA	490
35	GCTCTCCATC AGCTCCTGTC TCCAGCAGCT TTCCCTGTTG ATGTGGATCA CGCAGTGCTT	550
	TCTGCCCCGTG TTTTGGCTC AGCCTCCCTC AGGGCAGAGG CGCTAAGCCC AGCCTGGCGC	610
	CCCTTCCTAG GTCATGCCTC CTCCCCTAGG GAATGGTCCC AGCACGAGTG GCCAGTTCAT	670
40	TGTGGGGGCC TGATTGTTTG TCGCTGGAGG AGGACGGCTT ACATGTTTGT TTCTGTAGAA	730
	AATAAACTG AGCTACGAAA AA	752

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 58 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met	Leu	Met	Ala	Gln	Glu	Ala	Leu	Ala	Phe	Leu	Met	Ala	Gln	Gly	Ala
1				5					10				15		

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Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala Glu Val Pro  
20 25 30

5 Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu Ala Pro Arg  
35 40 45

Gly Val Arg Met Ala Ala Arg Leu Gln Gly \*  
50 55

10

## 2) INFORMATION FOR SEQ ID NO: 11:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
1 5 10....

25

## 2) INFORMATION FOR SEQ ID NO: 12:

30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
1 5 10.

40

## (2) INFORMATION FOR SEQ ID NO: 13:

45 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: synthetic DNA

GGTGACACTA TAGAAGGTAC G

21

55



(2) INFORMATION FOR SEQ ID NO: 14:

5 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: synthetic DNA

TGATGTGCAA CTGAAGCAGG.....20

15

(2) INFORMATION FOR SEQ ID NO: 15:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 21 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: synthetic DNA

GCACTGCGTG ATCCACATCA A 21

30

(2) INFORMATION FOR SEQ ID NO: 16:

35 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 21 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: synthetic DNA

CGACTCACTA TAGGGAGAGA G 21

45

(2) INFORMATION FOR SEQ ID NO: 17:

50 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 25 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: synthetic DNA

GCACATCAGC ATGCCTTTCT CGTCG

25

5

(2) INFORMATION FOR SEQ ID NO: 18:

10

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 32 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: synthetic DNA

CACACAAAGC TTGGCTTAGC GCCTCTGCCC TG.....32

20

(2) INFORMATION FOR SEQ ID NO: 19:

25

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: synthetic DNA

CACACAGGAT CCATGGATGC TGCAGATGCG.....30

35

(2) INFORMATION FOR SEQ ID NO: 20:

40

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: synthetic DNA

GAAGAACATA TGCTGATGGC CCAGGAGGC

29

50

55

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

TTAAAGATCT CAGAACCGCC CCTGGTCG

28

Claims

1. Tumor-associated antigen encoded by the ORF-1 of LAGE-1 cDNA and by the ORF-1 of cDNAs hybridizing with LAGE-1.
2. A tumor-associated antigen of claim 1 designated CAMEL which has the amino acid sequences set forth in SEQ ID NO: 2.
3. A tumor-associated antigen of claim 1, encoded by the ORF-1 of the NY-ESO-1 cDNA, the polynucleotide sequence of which is set forth in SEQ ID NO: 7.
4. A tumor-associated antigen of any of claims 1 to 3 for use in cancer therapy.
5. An immunogenic (poly)peptide derived from a tumor-associated antigen as defined in any one of claims 1 to 3.
6. The immunogenic (poly)peptide of claim 5, characterized in that it is derived from CAMEL.
7. The immunogenic (poly)peptide of claim 6, characterized in that it has the amino acid sequence Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu (SEQ ID NO: 11).
8. The immunogenic (poly)peptide of claim 6, characterized in that it has the amino acid sequence Leu Met Ala Gln Glu Ala Leu Ala Phe Leu (SEQ ID NO: 12).
9. The immunogenic (polypeptides) of any one of claims 6 to 8 for use in cancer immunotherapy.
10. A pharmaceutical composition containing an immunogenic (poly)peptide of any one of claims 6 to 8.
11. An isolated DNA molecule comprising the ORF-1 of LAGE-1 cDNA.
12. An isolated DNA molecule comprising the ORF-1 of a cDNA hybridizing with LAGE-1 under low stringency conditions.
13. An isolated cDNA molecule encoding CAMEL.
14. The isolated cDNA molecule of claim 13 which comprises nucleotides 54 - 336 of the sequence set forth in SEQ ID NO: 1.
15. An isolated DNA molecule comprising the ORF-1 of the NY-ESO-1 cDNA, which is set forth in SEQ ID NO: 9.

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16. Recombinant DNA molecule comprising a DNA molecule as defined in any one of claims 11-15.

17. A DNA molecule of any one of claims 11 to 16 for use in cancer immunotherapy.

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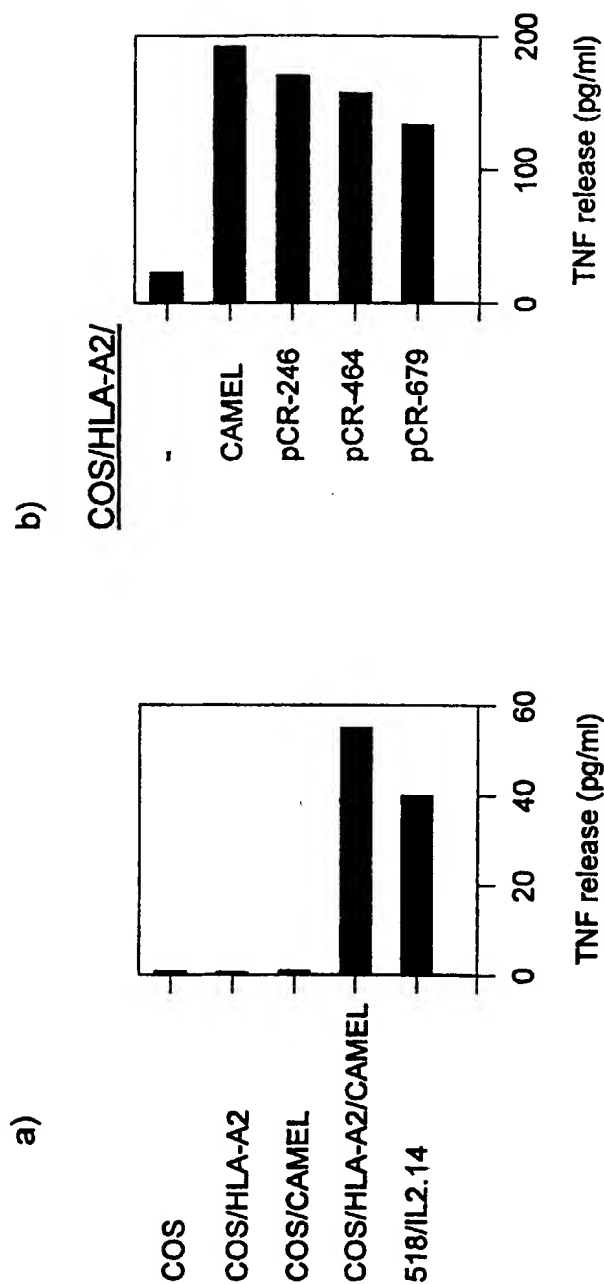
40

45

50

55

Fig. 1



30

Fig. 2 A continued

```

CAMEL                                     373
LAGE-1a                                -----
LAGE-1b                                -----
LAGE-1c                                -----
NY-ESO-1                                -----
GGGTGGTGGGTGGGGCTGGATCCGCTCCCGAGGGGAGAAAGCTCAGATCTCAGAACCCCAACAAGGTCTCAGAACAGAGACCTGGTAC
-----
                                     457
                                     600
                                     457

CAMEL                                     385
LAGE-1a                                -----
LAGE-1b                                -----
LAGE-1c                                -----
NY-ESO-1                                -----
ACGAGGCCGCGCCACCCGAGGAGCCAGGGAGATGGGTGCAGAGGTGTCGCTTTAATGTGATGTTCTCTGCCCTCACAATTTAGCCGACTGACTGC
-----
                                     469
                                     700
                                     469
                                     *****

CAMEL                                     485
LAGE-1a                                -----
LAGE-1b                                -----
LAGE-1c                                -----
NY-ESO-1                                -----
TGCAGACACCGCCCAACTGCAAGCTCTCCATCAGCTCTGCTCTCCAGCAGCTTTCCCTGTGTGATGTGGATCACCGAGTGTCTTGCCCGTGTGTTTGGCT
-----
TGCAGACACCGCCCAACTGCAAGCTCTCCATCAGCTCTGCTCTCCAGCAGCTTTCCCTGTGTGATGTGGATCACCGAGTGTCTTGCCCGTGTGTTTGGCT
-----
TGCAGACACCGCCCAACTGCAAGCTCTCCATCAGCTCTGCTCTCCAGCAGCTTTCCCTGTGTGATGTGGATCACCGAGTGTCTTGCCCGTGTGTTTGGCT
-----
                                     569
                                     *****

CAMEL                                     585
LAGE-1a                                -----
LAGE-1b                                -----
LAGE-1c                                -----
NY-ESO-1                                -----
CAGGCTCCCTCAGGGCAGAGGGGCTAAGCCAGCCTGGGGCCCTTCCCTAGTTCATGCTCCTCCCTAGGGAAATGGTCCACGACGAGTGGCCAGTTCA
-----
CAGGCTCCCTCAGGGCAGAGGGGCTAAGCCAGCCTGGGGCCCTTCCCTAGTTCATGCTCCTCCCTAGGGAAATGGTCCACGACGAGTGGCCAGTTCA
-----
CAGGCTCCCTCAGGGCAGAGGGGCTAAGCCAGCCTGGGGCCCTTCCCTAGTTCATGCTCCTCCCTAGGGAAATGGTCCACGACGAGTGGCCAGTTCA
-----
CAGGCTCCCTCAGGGCAGAGGGGCTAAGCCAGCCTGGGGCCCTTCCCTAGTTCATGCTCCTCCCTAGGGAAATGGTCCACGACGAGTGGCCAGTTCA
-----
                                     669
                                     *****

CAMEL                                     679
LAGE-1a                                -----
LAGE-1b                                -----
LAGE-1c                                -----
NY-ESO-1                                -----
TTGTGGGGCCCTGATGTTGTGCTGGAGGAGCGGCTTACATGTTTGTGTTCTGTAGAAATAAAGCTGAGCTACGAAAAAATAAATAAATAAATAA
-----
TTGTGGGGCCCTGATGTTGTGCTGGAGGAGCGGCTTACATGTTTGTGTTCTGTAGAAATAAAGCTGAGCTACGAAAAAATAAATAAATAAATAA
-----
TTGTGGGGCCCTGATGTTGTGCTGGAGGAGCGGCTTACATGTTTGTGTTCTGTAGAAATAAAGCTGAGCTACGAAAAAATAAATAAATAAATAA
-----
                                     752
                                     *****

```

Fig. 2 B

Protein Translations

ORF3

LAGE-1 <sup>s</sup>	MQAEGQGTGGSTGDADPGGPGIPDGPNGAGGPGEGACATGGRGPRGAGAARASGPRGGAPRGPHGGAASQAQDGRCPGGA	80
LAGE-1 <sup>l</sup>	MQAEGQGTGGSTGDADPGGPGIPDGPNGAGGPGEGACATGGRGPRGAGAARASGPRGGAPRGPHGGAASQAQDGRCPGGA	80
NY-ESO-1	MQAEGRGTTGGSTGDADPGGPGIPDGPNGAGGPGEGACATGGRGPRGAGAARASGPRGGAPRGPHGGAASGLNGCCRCGA	80
LAGE-1 <sup>s</sup>	RRPDSRLQLLHITMPFSSPMEAEELVRRILSRDAAPLPRPGAVLKDFTVSGNLLFIRLTAADHRQIQLSISSSCLOQLSLIM	160
LAGE-1 <sup>l</sup>	RRPDSRLQLLHITMPFSSPMEAEELVRRILSRDAAPLPRPGAVLKDFTVSGNLLFIRLTAADHRQIQLSISSSCLOQLSLIM	160
NY-ESO-1	RGPESSRLIEFTLAMPEATPMEAEELARRSLAQDAPPLPVPGVLLKEFTVSGNLLTIRLTAADHRQIQLSISSSCLOQLSLIM	160
LAGE-1 <sup>s</sup>	WITQCFLPVFLAQAPSGQRR	180 aa, 18.2 kD
LAGE-1 <sup>l</sup>	<b>EGCARDLNTFKKRVSEQRCTGCPPPPPGCAQCGCCRGVATNNVTSAPHI</b>	210 aa, 21.1 kD
NY-ESO-1	WITQCFLPVFLAQAPSGQRR	180 aa, 18.2 kD

ORF1

LAGE-1 <sup>s</sup>	MLMAQEAALAFMAQCAMLAQERRVPRAAEVPGAQGGQPRGEEAPRGVMAVPLLRMEGAPAGPGGRTAACFSCTSR	80
LAGE-1 <sup>l</sup>	MLMAQEAALAFMAQCAMLAQERRVPRAAEVPGAQGGQPRGEEAPRGVMAVPLLRMEGAPAGPGGRTAACFSCTSR	80
NY-ESO-1	<b>MLMAQEAALAFMAQCAMLAQERRVPRAAEVPGAQGGQPRGEEAPRGVMAVPLLRMEGAPAGPGGRTAACFSCTSR</b>	58
LAGE-1 <sup>s</sup>	CLSRPWPKRSWSAGSCPGMPHLSPDQGRF	109 aa, 11.7 kD
LAGE-1 <sup>l</sup>	CLSRPWPKRSWSAGSCPGMPHLSPDQGRF	109 aa, 11.7 kD
NY-ESO-1		58 aa, 6.2 kD



Fig. 3

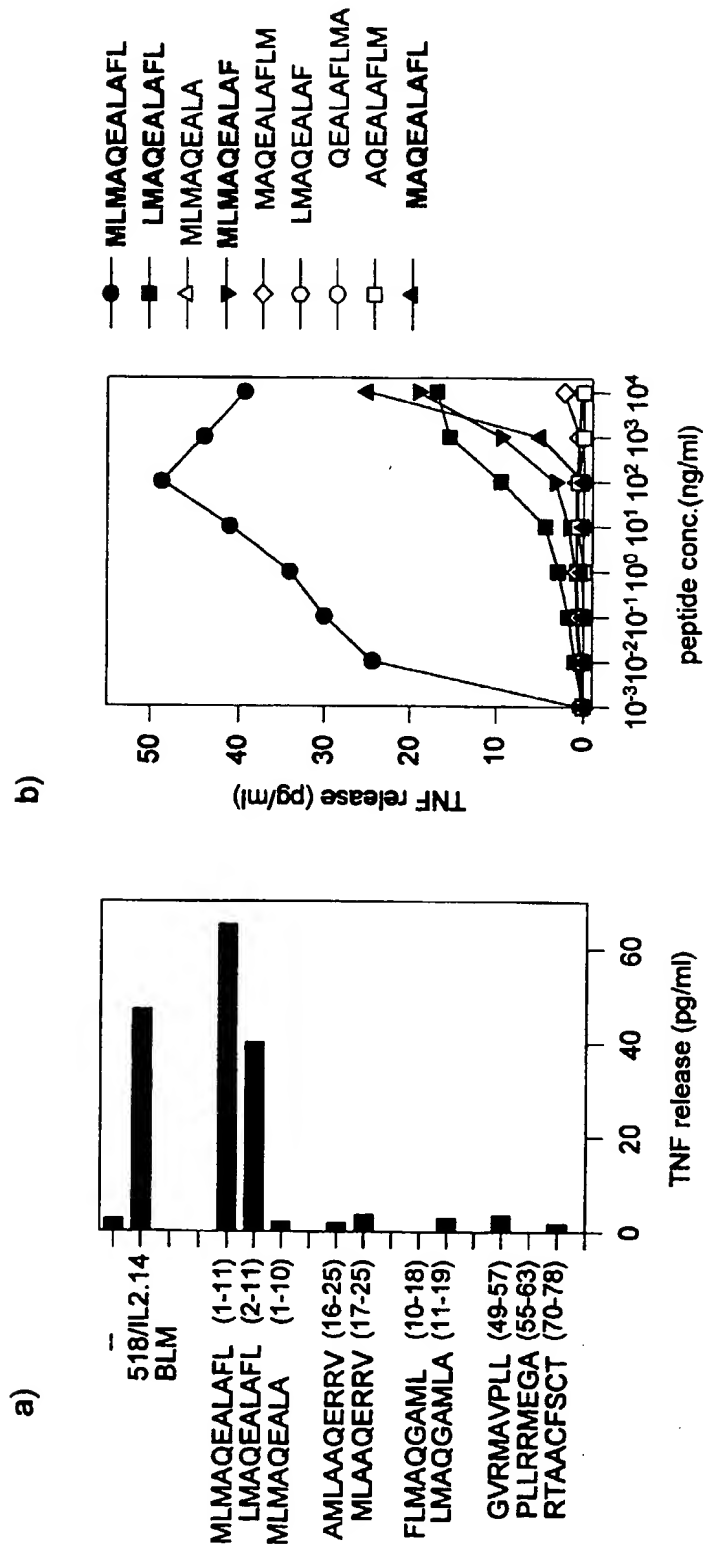


Fig. 4

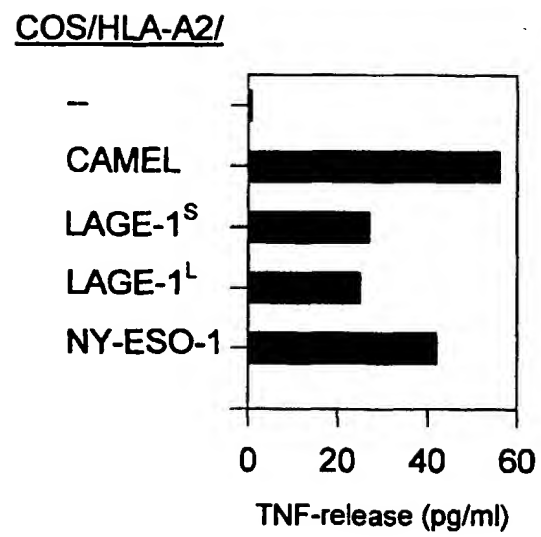


Fig. 5

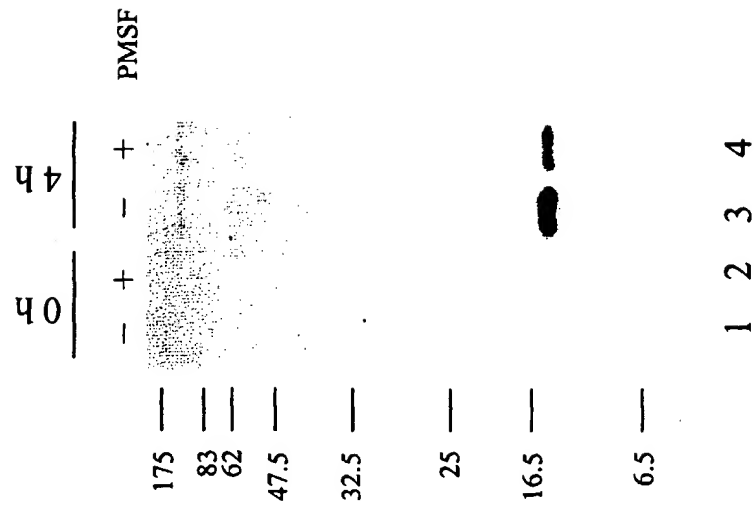


Fig. 6 A

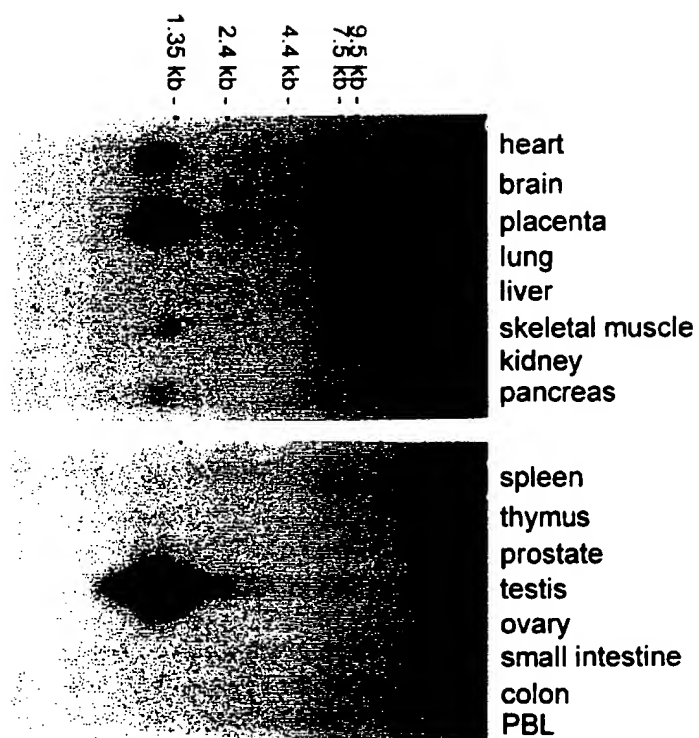
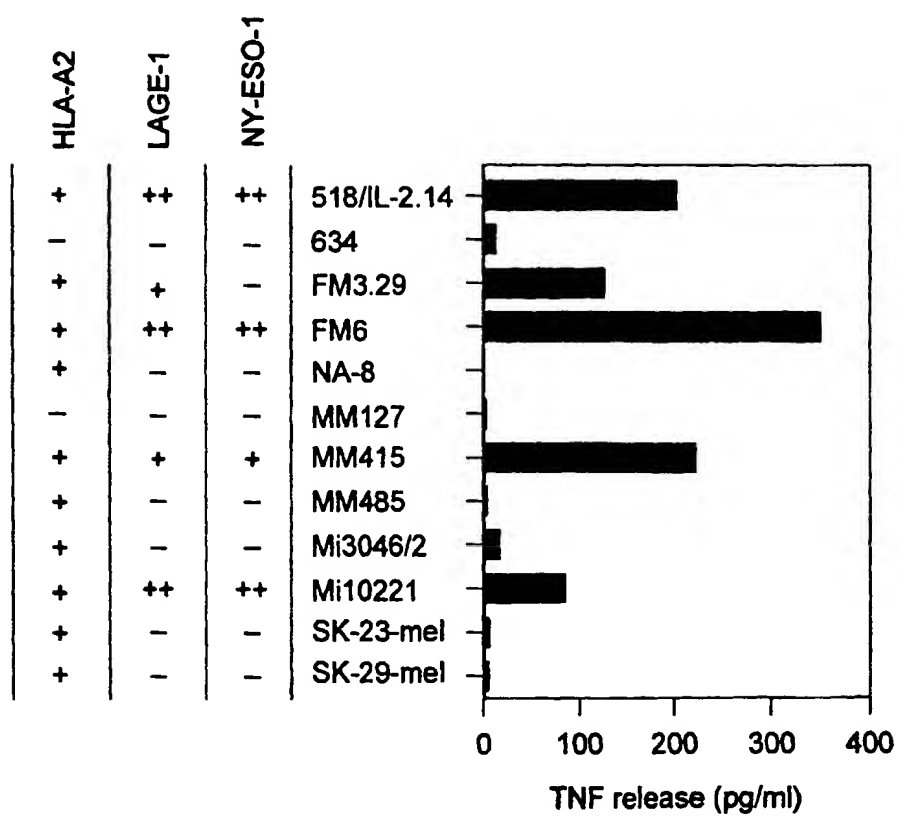


Fig. 6 B





European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 98 11 9583

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X	WO 98 32855 A (GODELAINE DANIELE ;LETHE BERNARD (BE); LUCAS SOPHIE (BE); SMET CHA) 30 July 1998 see whole document, particularly the claims	1,3-5, 11,12, 14-17	
A,D	VAN ELSAS, A. ET AL.: "Transfection of IL-2 augments CTL response to human melanoma cells in vitro: immunological characterization of a melanoma vaccine." JOURNAL OF IMMUNOTHERAPY, vol. 20, no. 5, September 1997, pages 343-53, XP002096030 * abstract; figures 6B,7 *		
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C07K A61K C12N
The present search report has been drawn up for all claims			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>10 March 1999</b>	Examiner <b>Smalt, R</b>
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

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ON EUROPEAN PATENT APPLICATION NO.**

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10-03-1999

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82